FEBS Letters 586 (2012) 798–803

journal homepage: www.FEBSLetters.org

Adaptive diversity of innate immune receptor family short pentraxins in Murinae

Yan Li [a,*], Judith H. Robins [b], Jianping Ye [c], Zhiyong Huang [d], Qiang Wen [a], Guojie Zhang [d,*]

[a] College of Animal Science and Technology, Sichuan Agriculture University, Yaan, Sichuan 625014, PR China
[b] School of Biological Sciences & Department of Anthropology, The University of Auckland, Private Bag 92019, Auckland, New Zealand
[c] College of Life Sciences, Shaoxing University, Shaoxing, Zhejiang 312000, PR China
[d] BGI-Shenzhen, Shenzhen 518083, PR China

ARTICLE INFO

Article history:
Received 14 November 2011
Revised 20 January 2012
Accepted 23 January 2012
Available online 1 February 2012

Edited by Takashi Gojobori

Keywords:
Short pentraxin
Adaptive diversity
Ligand-binding region
Subunit contact region
Murinae

ABSTRACT

The short pentraxins C-reactive protein (CRP) and serum amyloid P component (SAP) constitute a group of innate immune receptors that trigger immune activation by detecting molecules of the microbial cell wall. Here, we examined the evolution of short pentraxins in Murinae lineages. By molecular evolutionary analysis, *CRP* and *SAP* have been experiencing rapid diversification, driven by adaptive selection. Further, our protein modeling demonstrates that adaptively selected amino acids lie in the ligand-binding region and contact region between subunits. Our findings suggest that rapid diversification of these regions could contribute to the determinants of recognizing specificity and the interaction between subunits.

1. Introduction

The innate immune system is the first line of host defense against pathogens and is critical in activating and orienting adaptive immunity. The recognition of pathogens by receptors is the principle aspect of the innate immune system. The innate immune system recognizes pathogens through germline-encoded pattern recognition receptors (PRRs) [1].

Pentraxins are a superfamily of innate immune receptors characterized as having five or ten identical subunits, which are arranged in pentameric symmetry [2]. Based on the length of the subunits, the pentraxins are divided into two groups: long pentraxins and short pentraxins. PTX3 together with PTX4, NP1, NP2, and NPR constitute the long pentraxins. The short pentraxins include two members: CRP and SAP. CRP was the first pentraxin characterized in the 1930s and named for its ability to bind in a Ca^{2+}-dependent manner the C-polysaccharide of *Streptococcus pneumoniae*. Human SAP was subsequently identified as a relative of CRP because of the amino acid sequence similarity (51%) between them [3,4].

The crystal structures of human CRP and SAP have been determined, supporting that they are related [2,5,6]. The subunit (protomer) consists of a two-layered, antiparallel β sheet and a single, short α-helix with a flat topology. One face, designated A, is characterized by the presence of a furrow. Mutagenesis studies have demonstrated that certain residues in the furrow of CRP make contact with C1q [7–9] and the IgG receptors FcγRI (CD64) and FcγRII (CD32) [10–13]. The other face, designated B, contains a ligand-binding concave face. The ligand-binding site consists of several residues that form a hydrophobic pocket and two Ca^{2+} ions [14,15]. The contact domain between subunits comprises the open-core end of the two-layered β sheet of one protomer and the N- and C-terminal strands of another [16].

Short pentraxins recognize ligands and protect against various pathogens, including parasites, fungi, yeasts, and bacteria [17–19]. The direct interaction between CRP and phosphorylcholine (PC) mediates its binding to several microorganisms, including the C-polysaccharide of pneumococcus [20], the lipopolysaccharide (LPS) of *Hemophilus influenzae* [21], and the repeating phosphorylated disaccharide of *Leishmania donovani* [22]. Moreover, CRP binds carbohydrates on the surface of *Plasmodium falciparum*, *Hymenolepis diminuta*, and *L. donovani* in a Ca^{2+}-dependent manner [23–26]. Like CRP, SAP recognizes various microorganisms, such as *Streptococcus pyogenes*, *Neisseria meningitidis*, and the influenza virus [27–29]. The ligands for SAP include core polysaccharide moieties of bacterial LPS and phosphoethanolamine (PE) and chemical

Abbreviations: CRP, C-reactive protein; SAP, serum amyloid P component; d_N, the number of non-synonymous substitutions per non-synonymous site; d_S, the number of synonymous substitutions per synonymous site

* Corresponding authors. Fax: +86 835 2886080.
E-mail addresses: liyan@sicau.edu.cn (Y. Li), zhanggj@genomics.org.cn (G. Zhang).

0014-5793/$36.00
doi:10.1016/j.febslet.2012.01.048

components on lipid A [29–32]. The recognition of ligands by short pentraxins initiates a series of innate immune reactions, such as the activation of the classical complement pathway [33,34] and the stimulation of macrophages [12,13,35–38].

Pentraxins are generally regarded as a superfamily of evolutionarily conserved proteins characterized by their structural and physiological role in organisms. However, many previous studies have suggested that pathogens have evolved a range of anti-immune molecular mechanisms to overcome both innate and adaptive immunity [39]. In the case of the short pentraxins, there are conflicting reports of a possible protective role of CRP toward ligands. In the in vivo plasma clearance experiment, pneumococcal C-polysaccharide (CPS) a ligand to which CRP binds, was not cleared immediately in rats, indeed the half-life of the ligand was rather slower than it was in mice [40]. In another study, passively administered mouse CRP is unable to provide protection against the lethal toxicity of LPS [41]. In contrast to this, rabbit (*Oryctolagus cuniculus*) CRP expressed in mice is able to modulate a transgenic line to be resistant to the lethal effect of bacterial LPS [42]; and similarly, human CRP administration is protective in some experimental models [29,43]. Thus, determining the evolution of this gene family may be valuable in exploring the strategies by which hosts and pathogens compete in the arms race. To investigate the evolutionary process of short pentraxins in detail, we analyzed the nucleotide variation patterns of these receptors among *Rattus norvegicus*, *Mus musculus* and eight other species. This study might shed some light on the strategies of short pentraxins in the arms race between hosts and pathogens.

2. Materials and methods

2.1. Samples

To provide comprehensive information about the evolutionary patterns and processes of *CRP* and *SAP* in Murinae, we collected animals from nine species (*Apodemus agrarius*, *A. draco*, *M. caroli*, *M. pahari*, *Niviventer cremoriventer*, *R. exulans*, *R. norvegicus*, *R. rattus*, *R. tanezumi*). The sequences of *M. musculus* were obtained from GenBank (see below). The diversification events of these species have occurred between 12 and less than 0.3 My ago (Fig. S1). The species names and registry numbers are presented in Table S1.

2.2. Sequence and alignment

The entire *CRP* and *SAP* coding region of all samples was amplified from genomic DNA using primers in the conserved 5′ UTR and 3′ UTR regions. For *CRP*, the primers were: forward 5′-CTTAG TCCRGATCCCAGCAG-3′ and reverse 5′-CTCTAGTGCTGAGGACC-ACA-3′; for *SAP*, the primers were: forward 5′-GCTGC TGCTGTCA-TACCCTG-3′ and reverse 5′-AGGATTTTTATTTGGCAGATATG-3′. The PCR products were purified and cloned using the pMD 19-T Vector kit (TaKaRa). The inserts were sequenced using the vector primer. The sequences of a laboratory *M. musculus* strain (strain C57BL/6) and *R. norvegicus* (strain Sprague–Dawley), obtained from GenBank were used. Additional sequences of *CRP* and *SAP* genes in 32 individuals from five species ($n = 10$ *R. norvegicus*, 4 *R. tanezumi*, 10 *A. agrarius*, 4 *N. cremoriventer*, and 4 *M. caroli*) were determined with the same method, and the resulting sequences were aligned using ClustalW [44] and checked manually. The accession numbers of sequences are presented in Table S1.

2.3. Evolutionary analysis

To examine positive and negative selection *within* short pentraxins during Murinae evolution, we first analyzed the substitution patterns between pairs of species. Combining the sequences of *M. musculus* and *R. norvegicus*, we calculated the ratios of the number of non-synonymous substitutions per non-synonymous site (d_N) and the number of synonymous substitutions per synonymous site (d_S) for each coding region using Pamilo-Bianchi-Li's method [45]. The *Z*-test was used to detect deviations of d_N/d_S from neutrality.

After detecting adaptive selection in the coding regions, we examined positive selection at individual sites in the data set previously used in evaluating substitution patterns between species pairs (Table S1) with CODEML in the PAML package [46,47]. We implemented three pairs of models: M0 versus M2, M7 versus M8, and M8a versus M8, generating two likelihood ratios. M0, M7, and M8a assumed that all codons evolved neutrally or under purifying selection (d_N/d_S values $\leqslant 1$); M2 and M8 allowed a proportion of sites to be under positive selection with $d_N/d_S > 1$. In all tests, a likelihood ratio test (LRT) was used to compare the two models [48,49]. With the same maximum likelihood approach, we then analyzed allelic datasets of *CRP* and *SAP* from *R. norvegicus* and *A. agrarius* to assess whether positive selection had influenced the variation within species.

2.4. Modeling

Because no crystal structure of murine pentraxins have to date been reported, we simulated their tertiary structure with SWISS-MODEL (Automated Protein Modeling Server http://swissmodel.expasy.org/) [50,51]. The tertiary structure confidence (QMEAN4 *Z*-score) was assessed using Structure Assessment.

3. Results and discussion

3.1. The short pentraxins share the same gene structures in Murinae lineages

The molecular signature of adaptive selection can be identified through comparative sequence analysis among species. We amplified short pentraxin genes in eight species and cloned the products into vectors. One clone per species was sequenced and used to disclose the evolutionary patterns of the short pentraxin family in Murinae. The sequences had the same gene structures as *CRP* and *SAP* in *M. musculus* and *R. norvegicus*, based on their Ensembl entries.

3.2. Adaptive selection could have effects on the evolution of short pentraxins in Murinae lineages

Because the continual challenge posed by pathogenic diseases undoubtedly played an essential role in shaping the variation of present-day host immune systems, we analyzed the sequence variations in the entire coding region of *CRP* and *SAP* in these Murinae species. Across the *CRP* and *SAP* data sets, we observed a large number of non-synonymous substitutions. In the possible pairwise comparisons, the d_N/d_S ratios of *CRP* and *SAP* were calculated. For the *CRP* sequences, we observed that d_N exceeded d_S in *R. norvegicus* versus *N. cremoriventer* ($P = 0.039$) and *R. exulans* versus *N. cremoriventer* ($P = 0.046$) significantly. Further, the d_N/d_S ratios of *R. rattus* versus *N. cremoriventer*, *R. tanezumi* versus *N. cremoriventer*, *M. caroli* versus *N. cremoriventer*, and *A. agrarius* versus *A. draco* exceeded 1, which deviated from neutral expectation.

For the *SAP* sequences, using the *Z*-test, none of the pairwise comparison ratios exceeded one significantly. However, the ratios of *N. cremoriventer* to *R. norvegicus*, *R. rattus*, and *R. tanezumi* were similar to that of *CRP*, for which $d_N/d_S > 1$. In addition, the d_N/d_S ratios between *M. pahari* and *Rattus* greatly exceeded 1. These observations suggest that short pentraxins experienced Darwinian positive selection in Murinae lineages.

Table 1
Likelihood values and sites inferred to be under positive selection for short pentraxins genes across Murinae lineages.

Gene	Model	*l*	−2ΔL	Sites under selection identified by PAML
CRP				
	M0: one-ratio	−2225.62	41.70** (M2 versus M0)	None
	M2: selection	−2204.77		32 65 90 213 214 (at $0.95 > P \geqslant 0.9$)
				8 76 164 (at $0.9 > P \geqslant 0.8$)
	M7: beta	−2209.90	10.24** (M8 versus M7)	Not allowed
	M8a: beta & fixed ω	−2209.86	10.16** (M8 versus M8a)	Not allowed
	M8: beta & ω	−2204.78		32 65 76 90 213 214 (at $P \geqslant 0.95$)
				8 164 (at $0.95 > P \geqslant 0.9$)
				47 79 88 93 102 104 141 167 168 210 216 (at $0.9 > P \geqslant 0.8$)
SAP				
	M0: one-ratio	−2005.76	70.40** (M2 versus M0)	None
	M2: selection	−1970.56		26 159 (at $P \geqslant 0.99$)
				45 108 198 (at $P \geqslant 0.95$)
				105 (at $0.95 > P \geqslant 0.9$)
				9 25 167 187 (at $0.9 > P \geqslant 0.8$)
	M7: beta	−1983.83	26.46** (M8 versus M7)	Not allowed
	M8a: beta & fixed ω	−1983.78	26.36** (M8 versus M8a)	Not allowed
	M8: beta & ω	−1970.60		26 159 198 (at $P \geqslant 0.99$)
				25 45 105 108 (at $P \geqslant 0.95$)
				167 187 194 (at $0.95 > P \geqslant 0.9$)
				9 97 112 141 147 191 (at $0.9 > P \geqslant 0.8$)

Note. −2ΔL, twice the log likelihood difference between pair of models. Double asterisks correspond to $P < 0.01$ (one-tailed *t*-test). The numbers of amino acid sites represent their position in the interspecies sequences alignment.

Table 2
The amino acid substitutions at the adaptively selected sites of CRP and SAP across Murinae lineages.

Gene	Position	aa 1	aa 2	aa 3	aa 4	aa 5
CRP						
	32	Gly	Glu	Lys	Thr	
	65	Val	Ile	Ala		
	76	Lys	Thr	Glu	Asn	
	90	Gln	Glu	Lys		
	213	Thr	Ala	Lys	Val	
	214	His	Gln	Arg	Trp	
SAP						
	25	Asn	Asp	Lys	Tyr	
	26	Gln	Trp	Lys	Arg	Ser
	45	Trp	His	Arg		
	105	Leu	Lys	Arg	Met	Ile
	108	Phe	Tyr	Pro	His	
	159	Thr	Ser	Ala	Asn	
	198	Pro	Leu	Val	Ile	

Because interspecific and intraspecific comparisons could provide information about evolutionary patterns and processes acting over different timescales [52–54], we performed an allelic comparison of DNA sequence polymorphisms. We sequenced the coding region of *CRP* and *SAP* of 32 individuals per gene from five species ($n = 10$ *R. norvegicus*, 4 *R. tanezumi*, 10 *A. agrarius*, 4 *N. cremoriventer*, and 4 *M. caroli*). Notably, *CRP* and *SAP* harbored polymorphisms shared by different species (Fig. S1), with two amino acid-altering polymorphisms in each gene.

There are several possible sources that could account for the existence of interspecific shared polymorphisms, such as maintenance of trans-species polymorphisms, convergent evolution, and persistence of polymorphisms in recently diverged species. It is feasible that the same polymorphisms detected between any two species may have developed by chance when the species were young. Because some of these polymorphisms are present in both *Rattus* and *Apodemus* (Fig. S1), they have persisted in each species since their divergence 12–24 million years ago [55,56], strongly suggesting that the occurrence of such polymorphisms in these two genes is not due to an exceptionally young age of the species.

Because of the long time since divergence of these species from their common ancestor, it is difficult to decide between the role of convergent evolution and trans-species polymorphisms. The long-term maintenance of trans-species polymorphisms could be due to balancing selection, as shown for the major histocompatibility complex genes in jawed vertebrates [57–61]. Convergent change is common at amino acid sites that have been influenced by either positive selection [62–65] or relaxation of negative selection over long periods of time. In order to detect the evolutionary forces in this situation, we analyzed the within-species nucleotide variation in *R. norvegicus* and *A. agrarius* (see below).

Adaptive evolution typically occurs at few sites, as most amino acids in a protein are under functional constraints [45]. Using a codon-based test in a maximum likelihood framework [46,47], we narrowed down the individual sites that were under positive selection in *CRP* and *SAP* during Murinae evolution. We compared three pairs of models with and without positive selection using likelihood ratio tests, and found that in each comparison, the model that includes sites with $d_N/d_S > 1$ fits the data significantly better than the neutral one for both genes (Table 1). Both genes contained several sites for which $d_N/d_S > 1$ (Table 1). Six sites in *CRP* and seven sites in *SAP* had d_N/d_S ratios that rejected a neutral expectation in favor of adaptive selection ($P \geqslant 0.95$).

With the allelic datasets of *CRP* and *SAP* from *R. norvegicus* and *A. agrarius*, we repeated maximum likelihood analyses to assess whether positive selection had influenced the variation within species. For each of the genes, the proportion of sites under selection was relatively low (Table S2). However, three interspecific shared polymorphism sites showed $d_N/d_S > 1$ with a posterior probability of 0.8. These results suggested that natural selection played an important role in shaping these polymorphisms.

It is notable that some of the indicated amino acid changes at these sites are radical in terms of their striking physicochemical properties (Table 2). We used Grantham's distance, which relies on size, polarity, and carbon composition of amino acid side chains, to measure the underlying physicochemical differences between the exchanging residues [66]. Based on Grantham's distance, an amino acid substitution can be classified as either conservative or radical. Several amino acid changes at these sites were radical, strengthening the argument for a model of adaptive selection. For the *M. musculus*/*R. norvegicus* comparison, there were 4 putative selected sites in CRP that were different, of which the change at site 32 was radical with regard to its physicochemistry (Lys32 in

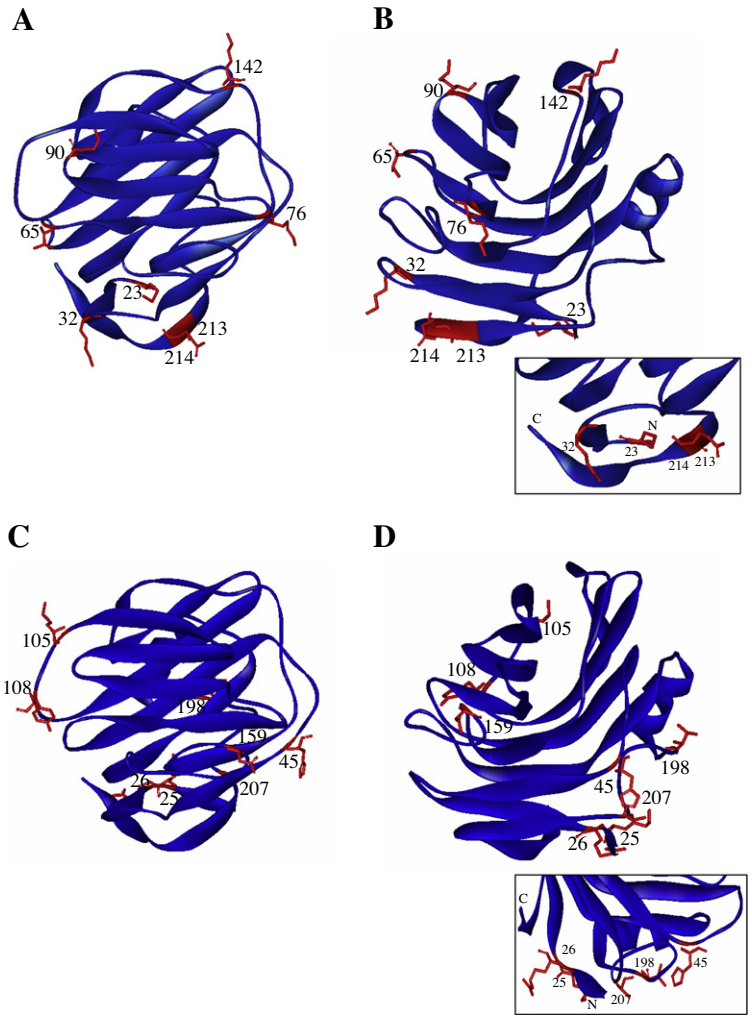

Fig. 1. Stick model showing the locations of adaptively selected amino acid sites in short pentraxins. The positions of adaptively selected amino acids are shown on the predicted three-dimensional structures of *R. norvegicus* CRP (A and B) and SAP (C and D) pentamers in red. (A and C): bottom view of the concave face for ligand-binding and nearby selected residues (65, 76, and 90 of CRP in (A); 105, 108, and 159 of SAP in (C)). (B and D): side view of the selected amino acids. In each case, the surfaces that associate to form a pentamer and contain sites under selection are shown in the squares. There are 21 amino acids missing in CRP and 20 amino acids missing in SAP compared with the template.

M. musculus and Gly32 in *R. norvegicus*, Grantham's distance: 127). Similarly, all seven selected sites in SAP varied between *M. musculus* and *R. norvegicus*, of which the substitution at site 45 (45His in *M. musculus* and 45Trp in *R. norvegicus*, Grantham's distance: 115) was radical. Overall, these evolutionary changes in CRP and SAP might have contributed to the functional divergence of these innate immune receptors in murine species.

3.3. Sites under adaptive selection are located on the ligand-binding and subunit interaction region of the short pentraxins

To gain insight into the structural and functional significance of the sites with evidence of selection, we located them on the three-dimensional structures of CRP and SAP. The Murinae short pentraxins shared the greatest similarity with human CRP (PDB ID: 1b09C) and SAP (PDB ID: 3kqrB) proteins. Pairwise alignments of targets against their templates showed that they share high sequence identity (greater than 60%) (Fig. S2), which confirmed that the determined structures of the templates could be used for modeling. The reliability of modeling was assessed, and the resulting QMEAN4 *Z*-scores (−1.22 and −0.83, respectively) ensured that the predicted structures were valid [67].

On mapping the putatively selected sites onto the structural models of CRP and SAP (Fig. 1), we observed that they fall in disparate functional domains. One group of sites (65, 76, and 90) is located on the side walls of the concave face on face B in CRP, as are the sites 105, 108, and 159 in the corresponding domain of SAP (Fig. 1A and C). It has been reported that the major interaction of CRP with PC occurs between the phosphate group and bound calcium ions in the center of the concave face [6,14–16]. The remaining portion of the PC molecule extends from the center and runs along the surface of CRP [16]. The position of PC on the LPS affects the susceptibility to CRP in *H. influenzae* [68]. In addition to PC, short pentraxins bind to a wide variety of carbohydrates on microbial surfaces [19]. Thus, the distribution of variable sites in the side walls of the concave face may help to diversify the pathogenic selectivity of short pentraxins, allowing them to recognize disparate ligands. In a comparison of the CPR sequences, we found that the residues at positions 65 and 90 in *M. musculus* differed from those in *R. norvegicus* (Fig. S3), which could contribute to the different plasma clearance rate of CPS in these species [40]. Further, comparing the CRP sequences of *M. musculus*, *Homo sapiens*, and *O. cuniculus*, we found that *O. cuniculus* and *H. sapiens* share the same residues at position 65 and 90, which are different from that

of *M. musculus* (Fig. S3). The differences among these residues are consistent with the phenomenon that the CRPs from *M. musculus* and the two other species provide the innate immune system's distinct specificity to LPS [29,41–43].

Another group of sites: 23, 32, 142, 213, and 214 in CRP and sites 25, 26, 45, 198, and 207 in SAP, lie in the interaction region of the protomer (Fig. 1B and D). In particular, sites 142 and 214 of Murinae CRP corresponded to sites 123 and 197 in the human ortholog, which form an intermolecular ion pair [16]. Two sites adopt different strategies: the shared polymorphisms (Lys and Glu) in site 142 presented striking physicochemical properties, and rapid changes at site 214 overlapped basic, non-polar, and uncharged polar residues. Interestingly, with regard to site 14 in the N-terminus of SAP, all four interspecific shared polymorphism sites fell in the interaction region (Fig. S1 and Fig. 1). In addition, amphipathic amino acids appeared frequently at these sites. One potential explanation for this is that the coexistence of polymorphisms and the frequent presence of amphipathic amino acids provide greater physicochemical variability for interacting residues in the adjacent protomer. Because the structural organization of protomers could play an important role in the activation of complement pathway [16,69], it is possible that such conversions constitute a functional adaptation that counters microbial insults that block the interaction between protomers [39]. Future functional studies are needed to determine the effects of these amino acid variations. If the hypothesis is supported, it might reveal more insights into the mechanism of the arms race between hosts and pathogens, as well as the coevolution of proteins and their endogenous interaction partners.

The above analysis provides evidence that adaptive selection drove the diversification at a number of sites, which were distributed on the different functional domains of short pentraxins. Understanding the evolution of these immune receptors in Murinae could provide important insights regarding the physiological function of these receptors and the mechanisms of the arms race between host immune systems and diverse pathogens.

Acknowledgments

We thank Scott Edward for his helpful comments and English editing of the manuscript. We thank Yu-Teh Kirk Lin for providing the *M. caroli* samples; Annie Orth for providing the *M. pahari* sample; Vernon Tintinger for providing the *R. exulans*, *R. rattus*, and *R. tanezumi* DNA samples; and Huailiang Xu and Yongfang Yao for providing the equipment. This work was supported by Scientific Research Fund of Sichuan Provincial Education Department (Grant No. 10ZA058 to Y.L.).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2012.01.048.

References

[1] Janeway Jr., C.A. (1989) Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb Symp Quant Biol 54 (Pt 1), 1–13.
[2] Shrive, A.K. et al. (1996) Three dimensional structure of human C-reactive protein. Nat Struct Biol 3, 346–354.
[3] Gewurz, H., Zhang, X.H. and Lint, T.F. (1995) Structure and function of the pentraxins. Curr Opin Immunol 7, 54–64.
[4] Rubio, N., Sharp, P.M., Rits, M., Zahedi, K. and Whitehead, A.S. (1993) Structure, expression, and evolution of guinea pig serum amyloid P component and C-reactive protein. J Biochem 113, 277–284.
[5] Emsley, J. et al. (1994) Structure of pentameric human serum amyloid P component. Nature 367, 338–345.
[6] Srinivasan, N., White, H.E., Emsley, J., Wood, S.P., Pepys, M.B. and Blundell, T.L. (1994) Comparative analyses of pentraxins: implications for protomer assembly and ligand binding. Structure 2, 1017–1027.
[7] Agrawal, A., Lee, S., Carson, M., Narayana, S.V., Greenhough, T.J. and Volanakis, J.E. (1997) Site-directed mutagenesis of the phosphocholine-binding site of human C-reactive protein: role of Thr76 and Trp67. J Immunol 158, 345–350.
[8] Agrawal, A., Shrive, A.K., Greenhough, T.J. and Volanakis, J.E. (2001) Topology and structure of the C1q-binding site on C-reactive protein. J Immunol 166, 3998–4004.
[9] Agrawal, A. and Volanakis, J.E. (1994) Probing the C1q-binding site on human C-reactive protein by site-directed mutagenesis. J Immunol 152, 5404–5410.
[10] Bang, R., Marnell, L., Mold, C., Stein, M.P., Clos, K.T., Chivington-Buck, C. and Clos, T.W. (2005) Analysis of binding sites in human C-reactive protein for Fc{gamma}RI, Fc{gamma}RIIA, and C1q by site-directed mutagenesis. J Biol Chem 280, 25095–25102.
[11] Muller, H. and Fehr, J. (1986) Binding of C-reactive protein to human polymorphonuclear leukocytes: evidence for association of binding sites with Fc receptors. J Immunol 136, 2202–2207.
[12] Marnell, L.L., Mold, C., Volzer, M.A., Burlingame, R.W. and Du Clos, T.W. (1995) C-reactive protein binds to Fc gamma RI in transfected COS cells. J Immunol 155, 2185–2193.
[13] Bharadwaj, D., Stein, M.P., Volzer, M., Mold, C. and Du Clos, T.W. (1999) The major receptor for C-reactive protein on leukocytes is fcgamma receptor II. J Exp Med 190, 585–590.
[14] Agrawal, A., Simpson, M.J., Black, S., Carey, M.P. and Samols, D. (2002) A C-reactive protein mutant that does not bind to phosphocholine and pneumococcal C-polysaccharide. J Immunol 169, 3217–3222.
[15] Mikolajek, H., Kolstoe, S.E., Pye, V.E., Mangione, P., Pepys, M.B. and Wood, S.P. (2011) Structural basis of ligand specificity in the human pentraxins, C-reactive protein and serum amyloid P component. J Mol Recognit 24, 371–377.
[16] Thompson, D., Pepys, M.B. and Wood, S.P. (1999) The physiological structure of human C-reactive protein and its complex with phosphocholine. Structure 7, 169–177.
[17] Szalai, A.J. (2002) The antimicrobial activity of C-reactive protein. Microbes Infect 4, 201–205.
[18] Marnell, L., Mold, C. and Du Clos, T.W. (2005) C-reactive protein: ligands, receptors and role in inflammation. Clin Immunol 117, 104–111.
[19] Kishore, U., Agrawal, A., Singh, P.P., Bottazzi, B., Garlanda, C. and Mantovani, A. (2009) Pattern recognition by pentraxins in: Target pattern recognition in innate immunity (Back, N., Cohen, I.R., Kritchevsky, D., Lajtha, A. and Paoletti, R., Eds.), pp. 98–116, Springer, New York.
[20] Volanakis, J.E. and Kaplan, M.H. (1971) Specificity of C-reactive protein for choline phosphate residues of pneumococcal C-polysaccharide. Proc Soc Exp Biol Med 136, 612–614.
[21] Weiser, J.N., Pan, N., McGowan, K.L., Musher, D., Martin, A. and Richards, J. (1998) Phosphorylcholine on the lipopolysaccharide of *Haemophilus influenzae* contributes to persistence in the respiratory tract and sensitivity to serum killing mediated by C-reactive protein. J Exp Med 187, 631–640.
[22] Bodman-Smith, K.B., Mbuchi, M., Culley, F.J., Bates, P.A. and Raynes, J.G. (2002) C-reactive protein-mediated phagocytosis of *Leishmania donovani* promastigotes does not alter parasite survival or macrophage responses. Parasite Immunol 24, 447–454.
[23] Pritchard, D.G., Volanakis, J.E., Slutsky, G.M. and Greenblatt, C.L. (1985) C-reactive protein binds leishmanial excreted factors. Proc Soc Exp Biol Med 178, 500–503.
[24] Pied, S., Nussler, A., Pontent, M., Miltgen, F., Matile, H., Lambert, P.H. and Mazier, D. (1989) C-reactive protein protects against preerythrocytic stages of malaria. Infect Immun 57, 278–282.
[25] Culley, F.J., Harris, R.A., Kaye, P.M., McAdam, K.P. and Raynes, J.G. (1996) C-reactive protein binds to a novel ligand on *Leishmania donovani* and increases uptake into human macrophages. J Immunol 156, 4691–4696.
[26] Taylor, K. and Hoole, D. (1997) Interactions between rat C-reactive protein and adult *Hymenolepis diminuta*. Parasitology 115 (Pt 3), 297–302.
[27] Hind, C.R., Collins, P.M., Baltz, M.L. and Pepys, M.B. (1985) Human serum amyloid P component, a circulating lectin with specificity for the cyclic 4,6-pyruvate acetal of galactose. Interactions with various bacteria. Biochem J 225, 107–111.
[28] Andersen, O., Vilsgaard Ravn, K., Juul Sorensen, I., Jonson, G., Holm Nielsen, E. and Svehag, S.E. (1997) Serum amyloid P component binds to influenza A virus haemagglutinin and inhibits the virus infection in vitro. Scand J Immunol 46, 331–337.
[29] Noursadeghi, M., Bickerstaff, M.C., Gallimore, J.R., Herbert, J., Cohen, J. and Pepys, M.B. (2000) Role of serum amyloid P component in bacterial infection: protection of the host or protection of the pathogen. Proc Natl Acad Sci USA 97, 14584–14589.
[30] de Haas, C.J., van der Tol, M.E., Van Kessel, K.P., Verhoef, J. and Van Strijp, J.A. (1998) A synthetic lipopolysaccharide-binding peptide based on amino acids 27–39 of serum amyloid P component inhibits lipopolysaccharide-induced responses in human blood. J Immunol 161, 3607–3615.
[31] Christner, R.B. and Mortensen, R.F. (1994) Binding of human serum amyloid P-component to phosphocholine. Arch Biochem Biophys 314, 337–343.
[32] Omtvedt, L.A., Wien, T.N., Myran, T., Sletten, K. and Husby, G. (2004) Serum amyloid P component in mink, a non-glycosylated protein with affinity for phosphorylethanolamine and phosphorylcholine. Amyloid 11, 101–108.
[33] Kaplan, M.H. and Volanakis, J.E. (1974) Interaction of C-reactive protein complexes with the complement system. I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin and sphingomyelin. J Immunol 112, 2135–2147.

[34] Siegel, J., Rent, R. and Gewurz, H. (1974) Interactions of C-reactive protein with the complement system. I. Protamine-induced consumption of complement in acute phase sera. J Exp Med 140, 631–647.
[35] Evans Jr., T.C. and Nelsestuen, G.L. (1995) Dissociation of serum amyloid P from C4b-binding protein and other sites by lactic acid: potential role of lactic acid in the regulation of pentraxin function. Biochemistry 34, 10440–10447.
[36] Gershov, D., Kim, S., Brot, N. and Elkon, K.B. (2000) C-Reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an antiinflammatory innate immune response: implications for systemic autoimmunity. J Exp Med 192, 1353–1364.
[37] Hicks, P.S., Saunero-Nava, L., Du Clos, T.W. and Mold, C. (1992) Serum amyloid P component binds to histones and activates the classical complement pathway. J Immunol 149, 3689–3694.
[38] Ying, S.C., Gewurz, A.T., Jiang, H. and Gewurz, H. (1993) Human serum amyloid P component oligomers bind and activate the classical complement pathway via residues 14–26 and 76–92 of the A chain collagen-like region of C1q. J Immunol 150, 169–176.
[39] Finlay, B.B. and McFadden, G. (2006) Anti-immunology: evasion of the host immune system by bacterial and viral pathogens. Cell 124, 767–782.
[40] Baltz, M.L., Rowe, I.F. and Pepys, M.B. (1985) In vivo turnover studies of C-reactive protein. Clin Exp Immunol 59, 243–250.
[41] Hirschfield, G.M., Herbert, J., Kahan, M.C. and Pepys, M.B. (2003) Human C-reactive protein does not protect against acute lipopolysaccharide challenge in mice. J Immunol 171, 6046–6051.
[42] Xia, D. and Samols, D. (1997) Transgenic mice expressing rabbit C-reactive protein are resistant to endotoxemia. Proc Natl Acad Sci USA 94, 2575–2580.
[43] Mold, C., Rodriguez, W., Rodic-Polic, B. and Du Clos, T.W. (2002) C-reactive protein mediates protection from lipopolysaccharide through interactions with Fc gamma R. J Immunol 169, 7019–7025.
[44] Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25, 4876–4882.
[45] Li, W.-H. (1997) Molecular evolution, Sinauer Associates, Sunderland, MA.
[46] Yang, Z. (1997) PAML: a program package for phylogenetic analysis by maximum likelihood. Comput Appl Biosci 13, 555–556.
[47] Yang, Z. (2007) PAML 4: phylogenetic analysis by maximum likelihood. Mol Biol Evol 24, 1586–1591.
[48] Nielsen, R. and Yang, Z. (1998) Likelihood models for detecting positively selected amino acid sites and applications to the HIV-1 envelope gene. Genetics 148, 929–936.
[49] Swanson, W.J., Nielsen, R. and Yang, Q. (2003) Pervasive adaptive evolution in mammalian fertilization proteins. Mol Biol Evol 20, 18–20.
[50] Schwede, T., Kopp, J., Guex, N. and Peitsch, M.C. (2003) SWISS-MODEL: an automated protein homology-modeling server. Nucleic Acids Res 31, 3381–3385.
[51] Arnold, K., Bordoli, L., Kopp, J. and Schwede, T. (2006) The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics 22, 195–201.
[52] Kimura, M. (1983) The neutral theory of molecular evolution, Cambridge University Press, Cambridge.
[53] Tajima, F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595.
[54] Wang, W., Thornton, K., Berry, A. and Long, M. (2002) Nucleotide variation along the *Drosophila melanogaster* fourth chromosome. Science 295, 134–137.
[55] Springer, M.S., Murphy, W.J., Eizirik, E. and O'Brien, S.J. (2003) Placental mammal diversification and the Cretaceous-Tertiary boundary. Proc Natl Acad Sci USA 100, 1056–1061.
[56] Adkins, R.M., Gelke, E.L., Rowe, D. and Honeycutt, R.L. (2001) Molecular phylogeny and divergence time estimates for major rodent groups: evidence from multiple genes. Mol Biol Evol 18, 777–791.
[57] Klein, J. and Figueroa, F. (1986) Evolution of the major histocompatibility complex. Crit Rev Immunol 6, 295–386.
[58] Cho, S., Huang, Z.Y., Green, D.R., Smith, D.R. and Zhang, J. (2006) Evolution of the complementary sex-determination gene of honey bees: balancing selection and trans-species polymorphisms. Genome Res 16, 1366–1375.
[59] Hughes, A.L. and Nei, M. (1988) Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. Nature 335, 167–170.
[60] Loisel, D.A., Rockman, M.V., Wray, G.A., Altmann, J. and Alberts, S.C. (2006) Ancient polymorphism and functional variation in the primate MHC-DQA1 5′ cis-regulatory region. Proc Natl Acad Sci USA 103, 16331–16336.
[61] Doherty, P.C. and Zinkernagel, R.M. (1975) Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. Nature 256, 50–52.
[62] Shen, Y.Y., Liu, J., Irwin, D.M. and Zhang, Y.P. (2010) Parallel and convergent evolution of the dim-light vision gene RH1 in bats (Order: Chiroptera). PLoS One 5, e8838.
[63] Li, Y., Liu, Z., Shi, P. and Zhang, J. (2010) The hearing gene Prestin unites echolocating bats and whales. Curr Biol 20, R55–R56.
[64] Liu, Y., Cotton, J.A., Shen, B., Han, X., Rossiter, S.J. and Zhang, S. (2010) Convergent sequence evolution between echolocating bats and dolphins. Curr Biol 20, R53–R54.
[65] Yeager, M. and Hughes, A.L. (1999) Evolution of the mammalian MHC: natural selection, recombination, and convergent evolution. Immunol Rev 167, 45–58.
[66] Grantham, R. (1974) Amino acid difference formula to help explain protein evolution. Science 185, 862–864.
[67] Benkert, P., Biasini, M. and Schwede, T. (2011) Toward the estimation of the absolute quality of individual protein structure models. Bioinformatics 27, 343–350.
[68] Lysenko, E., Richards, J.C., Cox, A.D., Stewart, A., Martin, A., Kapoor, M. and Weiser, J.N. (2000) The position of phosphorylcholine on the lipopolysaccharide of *Haemophilus influenzae* affects binding and sensitivity to C-reactive protein-mediated killing. Mol Microbiol 35, 234–245.
[69] Jiang, H., Robey, F.A. and Gewurz, H. (1992) Localization of sites through which C-reactive protein binds and activates complement to residues 14–26 and 76–92 of the human C1q A chain. J Exp Med 175, 1373–1379.